ties as a food collector, on account of local inertia and the total absence of tentacles, were supplemented by the life sustaining currents induced by its more active neighbors. These conditions are near Philadelphia furnished by *Urnatella gracilis* Leidy and *Pottsiella erecta* Kræpelin (*Paludicella erecta* Potts). I regret to be obliged to add that I am not aware that either of these has been collected in any other neighborhood.

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## SOME OBSERVATIONS ON THE PHYSIOLOGICAL FUNCTION OF THE PYLORIC CÆCA OF ASTERIAS VULGARIS.

By Ellen A. Stone.

The size and position of the pyloric cæca of our common star-fish, *Asterias vulgaris*, indicate an organ of great importance, yet their physiological function has been surprisingly little touched upon in any of the standard text-books of zoology. The most we can learn from them is a confused notion that they secrete some digestive fluid, which, according to some authors, is of unknown function, while others state that it is probably the representative of the bile of higher animals.

Dr. Griffiths and Dr. Fredericq, however, have demonstrated in the European species, *Uraster rubens*, the presence of active digestive ferments.[1] Their results, together with the abundant material and opportunity for studying these organs in Asterias, suggested the following experiments, which were carried on at the Laboratory of Physiological Chemistry, Brown University, under the direction of Mr. Ralph W. Tower.

The experimental methods and results are embodied in the following observations:

### A. Preliminary Examination of the Gland.

#### *I. The Reaction of the Secretion.*

Before making any examination for the ferment or ferments that might be contained in the secretion of the pyloric cæca,

[1] A. B. Griffiths, "Physiology of the Invertebrata," pp. 83-85.

the glands were first examined as to the nature of the reaction of this secretion, and this was found to be slightly acid since it produced a tinge of red in a neutral solution of litmus. This trace of acidity, however, was found to be due to some organic acid, since a neutral solution of tropaeolin 000, which is extremely sensitive to mineral acids, showed no change in color upon the addition of a small piece of the freshly crushed glands.

*II. Examination for Leucine.*

For the extraction of leucine, should any be present, the glands of several star-fishes were macerated with water and allowed to stand for some time. After filtering through cheese-cloth the filtrate was acidulated with acetic acid and boiled. A considerable coagulum of native albumen appeared, which was filtered off, and to the filtrate lead acetate added. The small precipitate was filtered off and sulphuretted hydrogen passed through the filtrate to remove the excess of lead. This precipitate was removed by filtration and the filtrate then evaporated to dryness. The residue was extracted with boiling alcohol, filtered and evaporated to a syrup. On standing, knots of crystals collected on the sides of the beaker which showed, by microscopic examination, the characteristics of very impure leucine. They could not, however, be certainly identified as such, since their quantity was insufficient to try Scherer's test. In all probability, however, if this method were applied to a number of glands sufficient to give a considerable yield of crystals, they could be purified and would undoubtedly prove to be leucine with probably some clusters of tyrosin.

B. Proof of the Non-Existence of Glycogen.

In determining whether the function of the pyloric cæca of the star-fish is more similar to that of the liver or to that of the pancreas of higher animals, one of the most reliable criteria would be the existence or non-existence of glycogen in the organ.

Therefore the cæca of about twenty living star-fishes were quickly removed and placed in boiling water where they were

allowed to boil for some time; the liquid was then filtered off and Pflüger's method for extracting glycogen[2] was carefully carried out. At the end of the process a portion of the resulting liquid gave with dilute iodine a very slight mahogany color, which seemed to indicate the presence of a little glycogen. But on boiling the remaining portion of the extract with dilute hydrochloric acid for half an hour, and then neutralizing with sodium hydrate, no substance was produced which would reduce Fehling's solution. The total amount of glycogen present in the glands of these twenty star-fishes must, therefore, have been very small, thus indicating that these organs do not possess a function characteristic of the true livers of higher animals. The following experiment also confirmed this view: Working upon the supposition that if glycogen were present in the pyloric cases, it would almost immediately be converted into glucose, the glands of several star-fishes were removed and allowed to stand in an open dish for about half an hour. At the end of that period a cold water extract was made of one portion while a hot water extract was made of another part. Both extracts were subjected to the test for glucose with Fehling's solution, and neither gave the slightest reduction, thus showing that the glands contained no glucose, and hence no antecedant of glucose in the form of glycogen.

### C. Examination for Digestive Ferments.

#### *I. Proteolytic Ferment.*

In carrying out this examination the proteid used was coagulated egg albumen, which was experimented upon in the following manner:

The white of one boiled egg was cut into small pieces and placed in 1000 cc. of a mixture of a 1 per cent. solution of sodium carbonate ($Na_2\ CO_3$) and sodium bi-carbonate ($NaHCO_3$) to which was added 100 cc. of a fresh water extract of the pyloric cæca of the star-fish and the whole was digested for 48 hours at a temperature of 37°C, the medium being kept free from bacteria by the presence of thymol. At the end of this

[2] Pflüger's Archiv. fur Physiologie, 1894, pp. 394–396.

period the albumen appeared considerably corroded and easily crumbled upon slight pressure between the fingers.

The substance was filtered and the filtrate then neutralized with dilute hydrochloric acid, upon which a slight precipitate of albuminate appeared. This was separated by filtration and the filtrate boiled, when a considerable coagulum of native albumen occurred. The native albumen was filtered off and the fluid then evaporated to about 300 cc.

To this concentrated fluid strong alcohol was added in considerable excess upon which a copious fine floculent precipitate of albumoses and peptones appeared which slowly settled to the bottom of the beaker. This precipitate was collected upon a filter and dissolved in 600–700 cc. of water. The precipitate completely dissolved, yielding an opalescent fluid which gave a strong xanthoproteic reaction and also a characteristic biuret test tending more strongly towards the rose-pink of a peptone. Ammonium sulphate was added to this solution in the form of crystals till the whole was completely saturated, and after standing for several hours a copious precipitate of albumoses gathered. This precipitate of albumoses was separated by filtration and the fluid was then treated with barium carbonate and barium hydroxide to precipitate the sulphate from the solution. After repeated treatments all the sulphate was finally precipitated and removed by filtration and the fluid was then boiled to a small bulk. About three volumes of strong alcohol were added and a fine floculent precipitate appeared which settled on standing. This precipitate was collected and dissolved in water, and the solution then gave the various characteristic reactions for peptones.

The first alcoholic filtrate was evaporated to a very small bulk, filtered and allowed to cool. After standing some time crystals of leucine and tyrosin were found upon microscopic examination, while the fluid also gave Hoffman's test for tyrosin.

At another time a set of parallel experiments was carried on in which equal amounts, by weight, of egg albumen were allowed to digest for an equal time with the same amounts of water extract of the glands, but under different circumstances.

Two portions were digested in a medium of 1 per cent. sodium carbonate and sodium bi-carbonate, the one at a temperature of 37° C., the other at a temperature of 21° C. Two other portions were digested in a medium of 3 per cent. Ditman's sea-salt solution; one at 37° C., the other at 21° C. Still a fifth portion was digested in a medium of 0.2 per cent. hydrochloric acid at 37° C. All media were kept free from bacteria by the presence of thymol. The native and derived albumins were removed in each case in the same manner and then the fluids were all evaporated to the same small bulk, 200 cc. To each, 600 cc. strong alcohol were added, and the albumoses and peptones thus precipitated were collected upon balanced filter papers and weighed in order to determine the relative amounts formed in the different digestive processes. The results showed that digestion had gone on most rapidly in the medium of sodium carbonate and sodium bi-carbonate, and moreover that it had gone on in this medium at a temperature of 37° C. more rapidly than at 21° C. This same relation held in the case of digestion in the sea-salt medium, that being the more rapid which was allowed to go on at the higher temperature. In the case of digestion in the acid medium, scarcely any albumoses and peptones were formed, showing that in so weak an acid medium even as 0.2 per cent, digestion was greatly retarded.

### *II. Diastatic Ferment.*

For this examination a dilute starch paste was made with 3 grams of starch to 200 cc. of water. To each of two portions of this paste was added an equal amount of fresh water extract of the pyloric cæca. Both were allowed to digest, one at a temperature of 37° C., the other at 21° C. Within fifteen minutes the substance digesting at 37° C. showed, upon testing with iodine, the port wine color peculiar to dexterine and also reduced the copper of Fehling's solution. Within half an hour these same reactions were produced in the portion of starch paste digesting at 21° C. Somewhat later both portions showed the presence of maltose in giving a negative test with iodine and a reduction of Fehling's solution. In this

case, too, that portion which was digesting at the higher temperature showed the presence of maltose first.

The digestive process converted the starch no further than maltose, for even after digesting all night no test for glucose was obtained by Barfold's test (acidified copper acetate solution).

### *III. Fat-Splitting Ferment.*

The presence of a fat-splitting ferment in the secretions of the pyloric cæca was proved by the fact that neutral olive oil, after being digested in the presence of thymol for same time, with a fresh neutral water extract of these cæca at a temperature of 37½° C., gave a decided acid reaction with litmus paper, thus showing that the neutral olein had been converted into free fatty acid and glycerine. A control portion of olive oil *alone*, which was submitted to the same digesting process under the same conditions, showed no acidity at the end of the experiment.

To sum up the conclusions that the results of the foregoing experiments seem to warrant, it seems that the pyloric cæca of the star-fish have no properties whatsoever—except, perhaps, their size and possibly color—which entitle them to a comparison with the liver of higher animals. On the contrary, however, they may be said to be closely related to the pancreas of the higher animals. Their secretion is abundant and contains three ferments :—

1. A Proteolytic ferment comparable to trypsin which acts best in a slightly alkaline medium, to good advantage in a neutral solution, but scarcely at all in an acid medium ; converting proteids into diffusible peptones and breaking down some of these even further into amido acids, as leucine and tyrosine.

II. A Diastatic ferment comparable to the diastatic enzyme of the pancreas which acts quite rapidly upon starch, converting it through the dextrines into maltose.

III. A Fat-splitting ferment comparable to that of the pancreas which breaks fats into their fatty acids and glycerine.

Upon these few but important and well-established facts it seems necessary, then, to abandon the old ground of charac-

terizing the pyloric cæca of the star-fish as of unknown function, and to advance so far, at least, as to characterize them as important digestive glands, very similar in function to the pancreas of higher animals.

Laboratory of Physological Chemistry, Brown University, Providence, R. I.

## EDITOR'S TABLE.

The confusion in the popular mind in regard to what biologists mean by "acquired character" is shown in a recent article by Prof. Cesare Lombroso,[1] and more strikingly in an editorial, apropos of this same article, which appeared a short time ago in the *Boston Medical and Surgical Journal*.[2]

Lombroso cites a number of cases which he regards as furnishing proof of the inheritance of acquired characteristics. In speaking of the development of the well known mental traits of the modern Hebrews he says: "Here we have a series of acquired psychical characteristics which have become heredity. This, no doubt, is due to some extent to climatic influences—transportation to colder countries—but more particularly to selection by persecution, as only by activity and the appearance of meanness and sordidness could the Hebrews have been saved from the fierce persecutions against which bold resistance would have been of no avail. It is this fact that made these vices prevail, and that caused the extinction, little by little, of those qualities—courage, generosity and boldness—that would have been more harmful than useful under the particular conditions." Again, in speaking of American traits of character, Lombroso says: "It happens because a race among the most robust of Europe has been transported to different surroundings; and the struggle for existence—rendered fiercer in the wilderness and among hostile tribes—if it served to destroy the weaker, gave room for the greater development of the strong, in whom qualities, perhaps already existent in the pacific Briton, but not yet unfolded for lack of occasion, emerged in the new adaptations required for new adventures." One might almost suppose these sentences to have been

[1] Lombroso, C.: The Heredity of Acquired Characteristics. *The Forum*, October, 1897, pp. 200–208.

[2] *Boston Medical and Surgical Journal*, October 21, 1897, Vol. 137, No. 17, p. 427.